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I, KAY WARD, TEAM LEADER EXAMINATION SUPPORT & SALES hereby certify that annexed is a true copy of the Provisional specification in connection with Application No. PQ0425 for a patent by UNIVERSITY OF SYDNEY filed on 18 May 1999.

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Sixteenth day of February 2000

K. Ward

KAY WARD
TEAM LEADER EXAMINATION
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University of Sydney

AUSTRALIA

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PROVISIONAL SPECIFICATION

for the invention entitled:

"An Assay-II"

The invention is described in the following statement:

of various genes. For example, some cancers are associated with changes in the expression of oncogenes and tumour suppressor genes. Furthermore, disease conditions or disorders associated with changes in cell cycle and development can be attributed to changes in transcriptional regulation of particular genes.

Although there are a number of genetic assays available to assess mutations, the identification of certain genetic changes cannot always be directly indicative of a disease condition or disorder.

Some genetic changes are expressed by alterations in cell surface antigens. Again, however, prior attempts to develop a diagnostic assay for complex disease conditions or disorders such as cancer based on the identification of a single antigen have not been universally successful.

Leukemias and lymphomas cause significant mortality and morbidity in humans. Such cancers result from the continuous proliferation of cells which would otherwise be blocked at various stages of normal differentiation to specialised cell types. Leukemias arise from blood forming cells in the bone marrow due to mutations in any of the precursors in the various lineages of differentiation (see Figure 1). Lymphomas develop from lymphocytes or macrophages in lymphatic tissue.

Lymphocytes in the peripheral blood express a large number of different antigens on their outer plasma membranes which are receptors for growth factors, cell-cell interactions and immunoglobulins; molecules for cell adhesion or complement stimulation; enzymes and ion channels. A single systematic nomenclature has been developed to classify monoclonal antibodies against human leukocyte cell surface antigens known as the cluster of differentiation (CD) antigens (Schlossman *et al,* 1995). Detailed information on CD antigens can be found at http://www.ncbi.nlm.nih.gov/prow/cd/index_molecules.htm. The expression of these cell-surface antigens can distinguish different types of mature blood cells found in the peripheral circulation.

Cells in the peripheral blood are produced in the bone marrow by proliferation and

requires the detection of the presence or absence of a range of antigens or quantification of their relative amounts. The resulting pattern of antigen expression is then indicative of the disease condition or disorder or a propensity for development of a disease or disorder.

SUMMARY OF THE INVENTION

One aspect of the present invention provides a diagnostic assay device comprising an array of molecules wherein in each molecule in the array, with the exception of a negative control, is capable of interaction with its respective binding partner putatively in a biological sample from an animal, avian species or plant wherein the pattern of interaction between the molecules and the binding partners is indicative of a disease condition or disorder or a propensity for the development of a disease condition or disorder.

Another aspect of the present invention contemplates an assay device for the diagnosis of cancer or a propensity for the development of cancer in an animal such as a human, said assay device comprising an array of molecules immobilized to a solid support wherein each molecule of the array, with the exception of a negative control, is capable of interaction with a respective binding partner if present in a biological sample from said animal wherein the pattern of interaction between the immobilized molecules and their respective binding partners is indicative of the presence of cancer or a propensity to develop cancer.

A related aspect of the present invention provides an assay device for the diagnosis of a non-neoplastic disorder or a propensity for the development of a non-neoplastic disorder in an animal such as a human, said assay device comprising an array of molecules immobilized to a solid support wherein each molecule of the array, with the exception of a negative control, is capable of interaction with a respective binding partner if present in a biological sample from said animal wherein the pattern of interaction between the immobilized molecules and their respective binding partners is indicative of the presence of a non-neoplastic disorder or a propensity to develop a non-neoplastic disorder.

A further aspect of the present invention is directed to an array of molecules immobilized on

that different discrete regions have specificity for different antigens and wherein the antigens are expressed on the surface of non-neoplastic cells or are released by non-neoplastic cells wherein the binding pattern of the immobilized immunoglobulins to their respective antigens is indicative of the presence of a non-neoplastic disorder or a propensity to develop a non-neoplastic disorder.

Another aspect of the present invention is directed to an array of immunoglobulins or derivatives or chemical equivalents thereof specific for antigens expressed on normal cells or cancerous cells or released by normal cells or cancerous cells wherein each group of immunoglobulins specific for each antigen or part thereof occupies a discrete region of a solid support, said array defined by the formula:

$$\left[\left[q_{o_1} \right]_e^{m_1} \left[q_{o_2} \right]_f^{m_2} \cdots\cdots \left[q_{o_k} \right]_g^{m_i} \right]_y$$

wherein

q is an immunoglobulin specific for an antigen expressed on a normal cell or cancerous cell or antigen released by a normal cell or cancerous cell;

m_1 m_2 ..... m_i represent members of the same immunoglobulin group which bind to different parts of the same antigen;

o_1 o_2 ..... o_k represent different groups of immunoglobulins defined by specificity to different antigens.

e, f and g represent the number of different immunoglobulins within each of groups o_1 o_2 ..... o_k, respectively and wherein e, f and g may be the same or different and each is from 0 to 100 provided that at least one of e, f and g is not 0;

y is the number of groups of immunoglobulins on the array and is from about 1 to about 2000;

wherein the pattern of interaction between the immobilized immunoglobulins and their respective antigens is indicative of the development of cancer or a propensity to develop

equivalents thereof immobilized to discrete regions of the solid support such that different discrete regions have specificity for different cluster of differentiation antigens and/or myeloid antigens expressed on leukemic cells wherein the binding pattern of the immobilized immunoglobulins to their respective antigens is indicative of the presence of cancer or a propensity to develop cancer.

Another aspect of the present invention provides a diagnostic assay device comprising an array of molecules wherein in each molecule in the array, with the exception of a negative control, is capable of interaction with its respective binding partner putatively in a biological sample from an animal, avian species or plant wherein the molecules are in an arrangement in said array such that upon interaction between the molecules and the binding partners a differential pattern of density provides an identifiable signal which is indicative of a disease condition or disorder or a propensity for the development of a disease condition or disorder.

A further aspect of the present invention contemplates an assay device for the diagnosis of cancer or a propensity for the development of cancer in an animal such as a human, said assay device comprising an array of molecules immobilized to a solid support wherein each molecule of the array, with the exception of a negative control, is capable of interaction with a respective binding partner if present in a biological sample from said animal wherein the molecules are in an arrangement in said array such that upon interaction between the immobilized molecules are their respective binding partners a differential pattern of density provides an identifiable signal which is indicative of the presence of cancer or a propensity to develop cancer.

Yet another aspect of the present invention is directed to an array of molecules immobilized on a solid support said array defined by the formula:

$$\left[\left[Px_1\right]_b^{n_1} \left[Px_2\right]_c^{n_2} \ldots\ldots \left[Px_j\right]_d^{n_j} \right]_z$$

$$\left[\left[q_{o_1}\right]_{e}^{m_1} \left[q_{o_2}\right]_{f}^{m_2} \cdots\cdots \left[q_{o_k}\right]_{g}^{m_i} \right]_{y}$$

wherein

q is an immunoglobulin specific for an antigen expressed on a normal cell or cancerous cell or antigen released by a normal cell or cancerous cell;

m_1 m_2 ..... m_i represent members of the same immunoglobulin group which bind to different parts of the same antigen;

o_1 o_2 ..... o_k represent different groups of immunoglobulins defined by specificity to different antigens.

e, f and g represent the number of different immunoglobulins within each of groups o_1 o_2 ..... o_k, respectively and wherein e, f and g may be the same or different and each is from 0 to 100 provided that at least one of e, f and g is not 0;

y is the total number of groups of immunoglobulins on the array and is from about 1 to about 2000;

wherein the molecules are in an arrangement in said array such that upon interaction between the immobilized immunoglobulins and their respective antigens a differential pattern of density provides an identifiable signal which is indicative of the development of cancer or a propensity to develop cancer.

Yet a further aspect of the present invention contemplates an assay device for cancer said device comprising an array of immunoglobulin molecules or functional derivatives or equivalents thereof immobilized to discrete regions of the solid support such that different discrete regions have specificity for different cluster of differentiation (CD) antigens and/or myeloid (MY) antigens expressed on leukemic cells wherein the molecules are in an arrangement in said array such that upon binding of the immobilized immunoglobulins to their respective antigens a differential pattern of density provides an identifiable signal which is indicative of the presence of cancer or a propensity to develop cancer.

o_1 o_2 ..... o_k represent different groups of immunoglobulins defined by specificity to different antigens;

e, f and g represent the number of different immunoglobulins within each of groups o_1 o_2 ..... o_k, respectively and wherein e, f and g may be the same or different and each is from 0 to 100 provided that at least one of e, f and g is not 0;

y is the total number of groups of immunoglobulins on the array and is from about 2 to about 2000;

wherein the molecules are in an arrangement in said array such that upon interaction between the immobilized immunoglobulins and their respective antigens a differential pattern of density provides an identifiable signal which is indicative of the development of cancer or a propensity to develop cancer.

Another aspect contemplates a method for determining the presence of a disease condition or disorder or a propensity to develop a disease condition or disorder such as but not limited to cancer in an animal, avian species or plant, said method comprising obtaining a biological sample from said animal, avian species or plant comprising free binding partners or binding partners bound to a cell surface, said binding partners associated directly or indirectly with said disease condition or disorder and contacting said biological sample with a solid support comprising an array of molecules capable of binding to said binding partners wherein the pattern of interaction with the binding partners is indicative of the disease condition or disorder or a propensity to develop said disease condition or disorder.

A further aspect of the present invention contemplates a method of detecting cancer or a propensity to develop cancer in a human or non-human animal, said method comprising obtaining a biological sample from said human or non-human animal and contacting said biological sample with an array of immunoglobulins or derivatives or chemical equivalents thereof specific for antigens expressed on normal cells or cancerous cells or released by normal cells or cancerous cells wherein each group of immunoglobulins specific for each antigen or part thereof occupies a discrete region of a solid support, said array defined by the formula:

the antigens are expressed on the surface of normal cells or cancerous cells or are released by normal cells or cancerous cells and determining the binding pattern of the immobilized immunoglobulins to their respective antigens and then undertaking immunotherapy such as with, but not limited to, humanized monoclonal antibodies based on the expression of the antigens.

BRIEF DESCRIPTION OF THE FIGURES

Figure 1 is a diagrammatic representation showing lineages for blood cell differentiation with precursor cell types from which various types of leukemias arise. Determination of complete antigen expression would enable unequivocal diagnosis of the leukemia (c.f. Figure 2). CML, Chronic Myeloid Leukemia; AMML, Acute Myelomonocytic Leukemia; ALL, Acute Lymphocytic Leukemia; AEL, Acute Erythrocytic Leukemia; AmegL, Acute Megakaryocytic Leukemia; AMoL, Acute Monocytic Leukemia; AML, Acute Myeloid Leukemia; CLL, Chronic Lymphocytic Leukemia; NHL, Non-Hodgkins Lymphoma; APL, Acute Promyelocytic Leukemia. Adapted from Cooper (1993).

Figure 2 is a diagrammatic representation showing lineages for differentiation of blood cells in the bone marrow. Some antigens expressed on precursor cells and mature blood cells are indicated. Adapted from van Dongen *et al* (1988).

Figure 3 is a diagrammatic representation of (a) a top view and (b) a perspective view of an immunoassay device comprising an array of discrete antibody spots.

Figure 4 shows Nomarski micrographs of human CCRF-CEM leukaemia and Raji lymphoma cells bound to some of the spots of an antibody (Coulter Beckman) array absorbed to a nitrocellulose film on a glass microscope slide (Molecular Probes). The procedures used are described in Examples 1 and 2, except that antibodies were applied in a volume of 0.4μL. A, CCRF-CEM cells; B, Raji cells.

invention is cancer in animals such as humans. The term "cancer" is used in its broadest sense and includes benign and malignant leukemias, sarcomas and carcinomas as well as other neoplasias. Neoplasias may be either malignant or benign. The cancers and neoplasias contemplated by the present invention may be simple (monoclonal, i.e. composed of a single neoplastic cell type), mixed (polyclonal, i.e. composed of more than one neoplastic cell type) or compound (i.e. composed of more than one neoplastic cell type and derived from more than one germ layer). Examples of simple cancers encompassed by the present invention include tumours of mesenchymal origin (e.g. tumours of connective tissue, endothelial tissue, blood cells, muscle cells) and tumours of epithelial origin. Particular cancers contemplated by the present invention include but are not limited to fibrosarcoma, myxosarcoma, Ewing's sarcoma, granulocytic leukemia, basal cell carcinoma, colon cancer, gastric cancer and a variety of skin cancers. Even more particularly, the cancers contemplated by the present invention include but are not limited to CML, Chronic Myeloid Leukemia; AMML, Acute Myelomonocytic Leukemia; ALL, Acute Lymphocytic Leukemia; AEL, Acute Erythrocytic Leukemia; AmegL, Acute Megakaryocytic Leukemia; AMoL, Acute Monocytic Leukemia; AML, Acute Myeloid Leukemia; CLL, Chronic Lymphocytic Leukemia; NHL, Non-Hodgkins Lymphoma and APL, Acute Promyelocytic Leukemia.

Accordingly, another aspect of the present invention contemplates an assay device for the diagnosis of cancer or a propensity for the development of cancer in an animal such as a human, said assay device comprising an array of molecules immobilized to a solid support wherein each molecule of the array, with the exception of a negative control, is capable of interaction with a respective binding partner if present in a biological sample from said animal wherein the pattern of interaction between the immobilized molecules and their respective binding partners is indicative of the presence of cancer or a propensity to develop cancer.

Another type of condition or disorder contemplated for detection in accordance with the present invention includes non-neoplastic disorders. A non-neoplastic disorder is considered herein to include any disorder or disease which is not characterized by uncontrolled cell proliferation and includes non-neoplastic disorders of the immune system. Such disorders include autoimmune disease including Type 1 diabetes, multiple sclerosis, psoriasis,

lymphocytes leading to death after approximately 11 years (Shinton, 1998). Treatment with zidovudine induces a transient increase in CD4+ lymphocytes; the effects of chemotherapy on various sub-sets of lymphocytes are proposed to be determined with an antibody array to monitor the clinical progression of HIV patients.

Immunophenotyping is also useful for bone marrow and organ transplantation for determination of the degree of immunosuppression and complications such as rejection or infection. Flow cytometry is used before transplantation for tissue typing using monoclonal antibodies to detect HLA-antigens, or for cross-matching. Tolerance to transplanted tissues is induced by administration of cyclosporin to the patient. Treatment at the time of transplantation forces the level of peripheral blood lymphocytes to almost zero. The disappearance of T cells from the blood is monitored by flow cytometry for CD3+ cells. Following transplantation, an increase in CD8+ cells signals possible rejection. Increases in CD8+ and CD16+, CD56+ and/or CD57+ cells may indicate viral infection. This surveillance of the immune state of the transplant patient is proposed to be done more rapidly and extensively using an antibody array of the present invention.

Some cancer patients receive high-dose chemotherapy and then require a bone marrow transplant to regenerate their depleted immune system. The transplanted marrow then induces haematopoietic reconstitution but unusual sub-sets of lymphocytes sometimes appear, such as CD5+ T cells. Opportunistic infections and graft-versus-host disease may occur in such patients resulting in an increase in CD8+ cells in both cases and an elevation of Natural Killer cells. Replacement of flow cytometry with an antibody array for CD antigens simplifies the monitoring of patients and provides more extensive information.

Antibody arrays for CD antigens would also be useful for monitoring changes in lymphocyte sub-sets during desensitisation therapy to insect or spider venom, for example, where the numbers of CD4+ CD45RA+ lymphocytes increase and CD4+ CD45RO+ lymphocytes decrease from elevated levels.

Patients with Chronic Fatigue Syndrome may have immunological abnormalities consistent

condition or disorder being diagnosed. In the case of cancer, for example, a particular cancer or the development of cancer in general may be determined on the basis of the expression of certain cell-surface ligands (and more particularly antigens) or the release of soluble ligands (e.g. soluble antigens). The P molecules are then selected as binding partners to the cell surface or soluble ligands. The pattern of interaction between the P molecules and their respective binding partners is indicative of the development of, or a propensity to develop, cancer or other disease conditions or disorders.

The array may also comprise a positive and/or negative control to assist in maintaining quality control of the assay procedure. A positive control, for example, may be a P molecule capable of interaction with a binding partner known to be present in the biological sample.

Another aspect of the present invention is directed to an array of molecules immobilized on a solid support said array defined by the formula:

$$\left[\left[Px_1\right]_b^{n_1} \left[Px_2\right]_c^{n_2} \ \ldots\ldots \ \left[Px_j\right]_d^{n_i} \right]_z$$

wherein

P is a member of a binding group capable of interacting with a binding partner;

$n_1\ n_2\ \ldots\ldots\ n_i$ represent different members of the binding group;

$x_1\ x_2\ \ldots\ldots\ x_j$ represent different binding groups;

b, c and d represent the number of different members of the binding groups $x_1\ x_2\ \ldots\ldots\ x_j$; respectively and wherein b, c and d may be the same or different and each is from about 0 to about 100 provided that at least one of e, f and g is not 0;

z is the total number of groups of molecules on the array and is from about 2 to about 2000;

wherein the pattern of interaction between the immobilized molecules and their respective binding partners is indicative of a disease condition or disorder or a propensity to develop

In a related embodiment, there is provided an assay device for a non-neoplastic disorder said device comprising an array of immunoglobulin molecules or functional derivatives or equivalents thereof immobilized to discrete regions of the solid support such that different discrete regions have specificity for different antigens and wherein the antigens are expressed on the surface of non-neoplastic cells or are released by non-neoplastic cells wherein the binding pattern of the immobilized immunoglobulins to their respective antigens is indicative of the presence of a non-neoplastic disorder or a propensity to develop a non-neoplastic disorder.

Generally, a range of immunoglobulins is selected on the basis of antigens expressed on normal cells, cancer cells and potential cancer cells. Each group of immunoglobulins specific for a different antigen is defined by $x_1\ x_2\ ....\ x_j$. Generally, a sample would contain a mixed population of cells and the immunoglobulin molecules on the array are designed to selectively bind to antigens on particular cell types.

Within each of groups $x_1\ x_2\ .....\ x_j$, a number of immunoglobulin sub-groups may exist specific for different parts of the same antigen defined by the groups $x_1\ x_2\ .....\ x_j$. These sub-groups are defined by $n_1\ n_2\ .....\ n_i$.

Accordingly, another aspect of the present invention is directed to an array of immunoglobulins or derivatives or chemical equivalents thereof specific for antigens expressed on normal cells or cancerous cells or released by normal cells or cancerous cells wherein each group of immunoglobulins specific for each antigen or part thereof occupies a discrete region of a solid support, said array defined by the formula:

$$\left[\left[q_{o_1}\right]_e^{m_1} \left[q_{o_2}\right]_f^{m_2} \ ..... \ \left[q_{o_k}\right]_g^{m_i} \right]_y$$

wherein

Preferably, the immunoglobulins are monoclonal antibodies but the present invention extends to polyclonal antibodies or antigen-binding parts, derivatives, homologues or analogues thereof as well as fusion or hybrid antibodies, synthetic antibodies or recombinant antibodies.

Still another aspect of the present invention provides an array of immunoglobulins or derivatives or chemical equivalents thereof specific for antigens expressed on non-neoplastic cells or released by non-neoplastic cells wherein each group of immunoglobulins specific for each antigen or part thereof occupies a discrete region of a solid support, said array defined by the formula:

$$\left[\left[q_{o_1} \right]_e^{m_1} \left[q_{o_2} \right]_f^{m_2} \cdots\cdots \left[q_{o_k} \right]_g^{m_i} \right]_y$$

wherein

q is an immunoglobulin specific for an antigen expressed on a lymphocyte or antigen released by a lymphocyte;

m_1 m_2 ..... m_1 represent members of the same immunoglobulin group which bind to different parts of the same antigen;

o_1 o_2 ..... o_k represent different groups of immunoglobulins defined by specificity to different antigens.

e, f and g represent the number of different immunoglobulins within each of groups o_1 o_2 ..... o_k, respectively and wherein e, f and g may be the same or different and each is from 0 to 100 provided that at least one of e, f and g is not 0;

y is the number of groups of immunoglobulins on the array and is from about 1 to about 2000;

wherein the pattern of interaction between the immobilized immunoglobulins and their respective antigens is indicative of the development of a non-neoplastic disorder or a propensity to develop a non-neoplastic disorder.

to about 15 mm^2. Generally, each region or spot is made up of immunoglobulins having a single distinct specificity. Specificity in this context is with respect to different antigens or different regions of the one antigen. The preferred number of immunoglobulin spots is from about 10 to about 1000. Most preferably, the immunoglobulins are arranged as duplicates.

The immobilized array of immunoglobulins is then contacted by a biological sample comprising cells, cell debris, cell extracts, tissue fluid, serum or blood after treatment to prevent clotting. Preferably, the sample would contain a mixed population of cells. The contact is for a time and under conditions sufficient for cells carrying particular antigens to be captured by the immobilized immunoglobulin or for free antigens to be captured by the immobilized immunoglobulins. The captured cells and antigens may then be detected by any convenient means such as biochemically, histochemically, immunologically or microscopically. Immunologic detection is particularly convenient. For example, a second immunoglobulin specific for a captured antigen or cell, labelled with a reporter molecule, may be added. The identification of the reporter molecule indicates that the antigen is captured. Alternatively, after the second immunoglobulin is added and it forms a complex with the captured antigen, an anti-immunoglobulin immunoglobulin labelled with a reporter molecule is added and the presence of a signal from the reporter molecule determined.

By "reporter molecule", as used in the present specification, is meant a molecule which, by its chemical nature, provides an analytical identifiable signal which allows the detection of immunoglobulin bound antigen. Detection may be either qualitative or quantitative. The most commonly used reporter molecules in this type of assay are either enzymes, fluorophores or radionuclide containing molecules (i.e. radioisotopes). In the case of an enzyme immunoassay, an enzyme is conjugated to the second or third immunoglobulin, generally by means of glutaraldehyde or periodate. As will be readily recognised, however, a wide variety of different conjugation techniques exist which are readily available to one skilled in the art. Commonly used enzymes include horseradish peroxidase, glucose oxidase, β-galactosidase and alkaline phosphatase, amongst others. The substrates to be used with specific enzymes are generally chosen for the production, upon hydrolysis by the corresponding enzyme, of a detectable colour change. It is also possible to employ

contacting said biological sample with an array of immunoglobulins or derivatives or chemical equivalents thereof specific for antigens expressed on normal cells or cancerous cells or released by normal cells or cancerous cells wherein each group of immunoglobulins specific for each antigen or part thereof occupies a discrete region of a solid support, said array defined by the formula:

$$\left[\left[q_{o_1}\right]_{e}^{m_1} \left[q_{o_2}\right]_{f}^{m_2} \cdots\cdots \left[q_{o_k}\right]_{g}^{m_i} \right]_{y}$$

wherein

q is an immunoglobulin specific for an antigen expressed on a normal cell or cancerous cell or antigen released by a normal cell or cancerous cell;

m_1 m_2 ..... m_i represent members of the same immunoglobulin group which bind to different parts of the same antigen;

o_1 o_2 ..... o_k represent different groups of immunoglobulins defined by specificity to different antigens.

e, f and g represent the number of different immunoglobulins within each of groups o_1 o_2 ..... o_k, respectively and wherein e, f and g may be the same or different and each is from 0 to 100 provided that at least one of e, f and g is not 0;

y is the total number of groups of immunoglobulins on the array and is from about 2 to about 2000;

wherein the pattern of interaction between the immobilized immunoglobulins and their respective antigens is indicative of the development of cancer or a propensity to develop cancer.

Yet another aspect of the present invention is directed to the use of an array of molecules capable of interaction with a respective binding partner putatively in a biological sample to determine the presence of a disease condition or disorder or a propensity for the development of a disease condition or disorder.

bound antigens, internal cellular antigens or soluble (i.e. free) antigens.

In a particularly preferred embodiment, the immunoglobulins are arranged on the array such that if a cancer being screened is present, the pattern of interaction provides a differential density which gives an identifiable shape, such as a letter, numeral or geometric design which can be readily detected.

For example, the antigens likely to be expressed during Acute Myeloid Leukemia (AML) can be arranged such that the letter "A" is preferentially shown as more dense dots relative to background. The letter "A" is used for the purposes of exemplification only, since any geometric shape or arrangement may be used.

Yet another aspect of the present invention is directed to an array of molecules immobilized on a solid support said array defined by the formula:

$$\left[\left[Px_1\right]_b^{n_1} \left[Px_2\right]_c^{n_2} \ldots\ldots \left[Px_j\right]_d^{n_i} \right]_z$$

wherein

P is a member of a binding group capable of interacting with a binding partner;

n_1 n_2 ..... n_i represent different members of the binding group;

x_1 x_2 ..... x_j represent different binding groups;

b, c and d represent the number of different members of the binding groups x_1 x_2 ..... x_j; respectively and wherein b, c and d may be the same or different and each is from about 0 to about 100 provided that at least one of b, c or d is not 0;

z is the total number of groups of molecules on the array and is from about 1 to about 2000;

wherein molecules are in an arrangement in said array such that upon interaction between the immobilized molecules and their respective binding partners, a differential pattern of density

e, f and g represent the number of different immunoglobulins within each of groups o_1 o_2 ..... o_k, respectively and wherein e, f and g may be the same or different and each is from 0 to 100 provided that at least one of e, f and g is not 0;

y is the total number of groups of immunoglobulins on the array and is from about 1 to about 2000;

wherein the molecules are in an arrangement in said array such that upon interaction between the immobilized immunoglobulins and their respective antigens, a differential pattern of density provides an identifiable signal which is indicative of the development of cancer or a propensity to develop cancer.

Still yet a further aspect of the present invention contemplates an assay device for cancer, said device comprising an array of immunoglobulin molecules or functional derivatives or equivalents thereof immobilized to discrete regions of the solid support such that different discrete regions have specificity for different cluster of differentiation (CD) antigens and/or myeloid (MY) antigens expressed on leukemic cells wherein the molecules are in an arrangement in said array such that upon binding of the immobilized immunoglobulins to their respective antigens a differential pattern of density provides an identifiable signal which is indicative of the presence of cancer or a propensity to develop cancer.

Another aspect contemplates a method for determining the presence of a disease condition or disorder or a propensity to develop a disease condition or disorder such as but not limited to cancer in an animal, avian species or plant, said method comprising obtaining a biological sample from said animal, avian species or plant comprising free binding partners or binding partners bound to a cell surface, said binding parties associated directly or indirectly with said disease condition or disorder and contacting said biological sample with a solid support comprising an array of molecules capable of binding to said binding partners wherein the molecules are in an arrangement in said array such that upon interaction with the binding partners a differential pattern of density provides an identifiable signal which is indicative of the disease condition or disorder or a propensity to develop said disease condition or disorder.

EXAMPLE 1
PREPARATION OF ANTIBODY ARRAY

The antibodies may be covalently linked to suitable nylon membrane such as an Immobilon P membrane (Millipore Corporation) without a requirement for subsequent blocking with an excess of a protein solution such as a skim milk preparation. The antibodies are also adsorbed to a nitrocellulose film on a glass microscope slide (Molecular Probes) and the unbound nitrocellulose is then blocked with skim milk. Antibodies are also adsorbed to Nylon membranes. To increase the accessibility of bound anti-CD antibodies to antigens on cells, the solid support used for the array is initially coated with a recombinant, truncated form of Protein G from *Streptococcus* which retains its affinity for IgG but lacks albumin and Fab binding sites, and membrane-binding regions (Goward *et al.*, 1990). Antibodies are applied to this coat of Protein G and bind *via* their Fc domains leaving the Fab domains free to interact with cells. The Fab domains are also further from the solid support providing greater accessibility of CD antigens on cell membranes to antibodies.

The array of antibodies is also constructed on a membrane or a coverslip. In this case, the antibodies are covalently linked to the membrane as duplicate spots in a two-dimensional matrix. The spots are arranged in a matrix such as but not limited to a 15 x 15 matrix. The antibodies are monoclonal and are specific for the cluster of differentiation (cluster designation) antigens (CD antigens) and myeloid (MY) antigens expressed on leukemia cells. Antibodies specific for LY antigens may also be included. Details of CD antigens are available at http://www.ncbi.nlm.nih.gov/prow/cd/index molecule.htm. The spots are of microscopic size and are produced by the application of a drop (~ 10 nanolitres) of antibody solution (e.g. 10 μg protein/ml) on designated portions of a membrane or glass surface such as a coverslip, first washed with a non-specific protein absorbent such as 30% w/v skim milk (Dutch Jug, Bonlac Foods Ltd, Melbourne, Australia) and then rinsed. Other protein solutions and other brands of skim milk may also be employed. The antibodies may be covalently coupled to the solid support such as through amino groups of lysine residues, the carboxylate groups of aspartate or glutamic acid residues or the sulfhydryl groups of cysteine residues. The array of antibodies selectively binds cells from body fluids which express the

EXAMPLE 3

ASSAY

Samples of cells or antigens are applied to the antibody-membrane array, incubated to enable maximal binding and non-bound material is then removed by a suitable washing solution. Generally, bound cells are fixed to the antibodies by chemical cross-linking. Cells bound to antibodies in the array are visualized microscopically to determine morphologies. Where necessary, cells are fixed to the array and stained, treated enzymically and/or tested for enzymic and/or receptor expression.

Fixed cells may also be interacted with a second antibody labelled with a reporter molecule (e.g. fluorochrome). Alternatively, a series of "second" antibodies are used each with a different fluorochrome. Expression of multiple antigens on cells is then determined by fluorescence confocal microscopy.

Fixed cells are also stained or fluorescently labelled to enable quantification of cell densities in the original body fluid sample. This quantification may be automated with a programmable scanner which records cell densities at each antibody dot in the array. For example, a laser densitometer (Molecular Dynamics) which scans in two dimensions (resolution 60 μm) is particularly useful. The degree of staining of the antibody spots is proportional to the number of cells bound to each antibody. For fluorescence detection, a FluorImager (Molecule Dynamics, Inc.) can be used (resolution 100-200 μM).

The antibody array such as against CD and MY antigens provides a pattern of expression of the antigens and this is then matched to set patterns of antigen expression for different leukemias (e.g. acute myeloid leukemia [AML]). For AML (M4), for example, the following antigens are expressed: MY4 (CD14), MY7, MY9 and M01 (CD11b).

intracellular esterases to form fluorescent 5-chloromethylfluorescein. This product undergoes a glutathione S-transferase–mediated reaction to produce a membrane-impermeant glutathione–fluorescent dye adduct which then reacts with thiols on proteins and peptides to form conjugates. Fluorescently-labelled cells bound to an antibody array are quantified using a scanning fluorimeter (e.g. STORM, Molecular Dynamics or FluorImager) or a confocal microscope. Mild reaction conditions are preferably used so that the majority of antigen binding sites are not affected. Different cells are labelled to different extents with different numbers of fluorophores. Cells are washed prior to reaction with the fluorophore.

(ii) Unlabelled cells bound to an array are reacted with soluble, fluorescently-labelled antibodies which bind to a different CD antigen on the cells enabling quantification of bound cells.

(iii) For some samples, the level of cellular CD antigen may exceed the number of antibodies available in a particular spot. CD antigens found to be expressed from an initial screen could be quantified subsequently using a row of dilutions of a particular antibody. When the number of antibodies in a spot on this array exceeded the number of CD antigens on cells applied in the sample, there is no further increase in fluorescence. A plot of fluorescence *versus* amount of antibody gives the density of cells expressing that CD antigen in the plasma sample. From these data, the number of a particular CD antigen per cell is calculated. Levels of bound cells on this quantitative array are determined using procedures (i) or (ii) above.

EXAMPLE 6
DETECTION OF FIXED CELLS

Bound cells on the antibody-membrane array are visualized microscopically to determine their morphologies. The cells are fixed to the array and stained, hybridized with a radioactively- or fluorescently-labelled oligonucleotide probe, treated enzymically or tested for enzymic activities, for determination of their morphologies. The fixed cells may also be reacted with a second labelled soluble antibody (for example with a fluorochrome attached), or indeed with a number

diagnosis may be automated with fluorometric or spectrophotometric scanning of the arrays to determine which antibody spots bound cells, with computerized recognition of patterns of antigen expression for particular cancers. This method enables automated diagnosis of a wide variety of leukemias, lymphomas and other metastatic cancers. Using a complete array of antibodies against CD and MY antigens, new types of leukemias and lymphomas may be discovered.

Identification of particular antigens on cancer cells provides essential information for subsequent immunotherapy of the patient with humanized monoclonal antibodies, or immunotoxins where a toxin or drug is covalently linked to the antibody. Progressive remission induced by chemotherapy and/or radiotherapy and subsequent relapse due to growth of drug-resistant cells is monitored in patients using periodic cell samples.

Other arrays of antibodies specific for antigens expressed by metastatic colon, breast, melanoma or other cancers could be made to detect very low levels of metastatic cells in body fluids (e.g. blood or cerebrospinal fluid) in patients with large primary tumours who would be at risk for metastasis. Such surveillance would be of particular importance for rapidly growing cancers such as melanomas.

A "user-friendly interface" may also be created with an antibody array by arranging antibodies which correspond to, for example, acute myeloid leukemia (AML) in the form of a character, say, "M" for this leukemia. The same antibody array could have antibodies for acute lymphocytic leukemia (ALL) arranged in an "L". In this way, a single array could give a direct diagnosis based upon known patterns of surface antigen expression for different cancers. The small size of the antibody dots and arrays of dots would enable several different tests to be performed on the same sample without significantly adding to the cost of the test. This is particularly true for 1-5 nanolitre-sized spots where each antibody would cost a small fraction of one cent.

A variety of non-neoplastic disorders of the immune system result in abnormal populations of circulating lymphocytes. Autoimmune diseases such as Type 1 diabetes, multiple sclerosis, myasthenia gravis, pernicious anaemia, psoriasis, rheumatoid arthritis, scleroderma and systemic

induces a transient increase in CD4+ lymphocytes; the effects of chemotherapy on various sub-sets of lymphocytes is monitored with an antibody array to follow the clinical progression of HIV patients.

Immunophenotyping is also useful for bone marrow and organ transplantation for determination of the degree of immunosuppression and complications such as rejection or infection (Bene and Martini, 1997). Flow cytometry is employed before transplantation for tissue typing using monoclonal antibodies to detect HLA-specificities, or cross-matching. Tolerance to transplanted tissues is induced by administration of cyclosporin to the patient. Treatment at the time of transplantation forces the level of peripheral blood lymphocytes to almost zero. The disappearance of T cells from the blood is monitored by flow cytometry for CD3+ cells. Following transplantation, an increase in CD8+ cells signals possible rejection and increases in CD8+ and CD16+, CD56+ and/or CD57+ cells may indicate viral infection. This surveillance of the immune state of the transplant patient is done more rapidly and extensively using an antibody array.

Some cancer patients receive high-dose chemotherapy and then require a bone marrow transplant to replace their depleted immune system. The transplanted marrow then induces haematopoietic reconstitution but the unusual sub-sets of lymphocytes sometimes appear, such as CD5+ T cells. Opportunistic infections and graft-versus-host disease may occur in such patients giving an increase in CD8+ cells in both cases and on elevation of Natural Killer cells. Replacement of flow cytometry with an antibody array for CD antigens simplifies the monitoring of patients and provides more extensive information.

Antibody arrays for CD antigens are also useful for monitoring changes in lymphocyte sub-sets during desensitisation therapy to wasp or bee venom where the numbers of CD4+ CD45RA+ increase and CD4+ CD45RO+ decrease from elevated levels. Patients with Chronic Fatigue Syndrome may have immunological abnormalities consistent with a viral infection with activated CD8+ lymphocytes. A detailed analysis of lymphocyte sub-sets by the antibody array of the present invention establishes new diagnostic criteria and provides fundamental information on this disorder.

defining new sub-sets of leukaemias based upon extensive information on antigen expression available using the single step assay.

Those skilled in the art will appreciate that the invention described herein is susceptible to variations and modifications other than those specifically described. It is to be understood that the invention includes all such variations and modifications. The invention also includes all of the steps, features, compositions and compounds referred to or indicated in this specification, individually or collectively, and any and all combinations of any two or more of said steps or features.

ASPECTS OF THE INVENTION INCLUDE BUT ARE NOT LIMITED TO:

1. A diagnostic assay device comprising an array of molecules wherein in each molecule in the array, with the exception of a negative control, is capable of interaction with its respective binding partner putatively in a biological sample from an animal, avian species or plant wherein the pattern or interaction between the molecules and the binding partners is indicative of a disease condition or disorder or a propensity for the development of a disease condition or disorder.

2. A diagnostic assay device according to aspect 1 wherein the biological sample is from a human or non-human animal.

3. A diagnostic assay device according to aspect 2 wherein the array comprises the formula:

$$\left[\left[Px_1\right]_b^{n_1} \left[Px_2\right]_c^{n_2} \ldots\ldots \left[Px_j\right]_d^{n_i} \right]_z$$

wherein

p is a member of a binding group capable of interacting with a binding partner;

n_1 n_2 ..... n_i represent different members of the binding group;

x_1 x_2 ..... x_j represent different binding groups;

b, c and d represent the number of different members of the binding groups x_1 x_2 ... x_j; respectively and wherein b, c and d may be the same or different and each is from about 0 to about 100 provided that at least one of b, c or d is not 0;

z is the total number of groups of molecules on the array and is from about 2 to about 2000.

4. A diagnostic assay device according to aspect 3 wherein the disease condition or disorder

8. A diagnostic assay device according to aspect 6 or 7 wherein the immunoglobulins are specific for cluster of differentiation (CD) antigens and/or myeloid (MY) antigens expressed on leukemic cells.

9. A diagnostic assay device according to aspect 3 wherein the disease condition or disorder is a non-neoplastic disorder.

10. A diagnostic assay device according to aspect 9 wherein the disease or condition is a non-neoplastic disorder of the immune system.

11. A diagnostic assay device according to aspect 9 or 10 wherein the disease or condition is selected from an autommine disease such as Type 1 diabetes, multiple sclerosis, myasthenia gravis, pernicious anaemia, psoriasis, rheumatoid arthritis, scleroderma and systemic lupus erythematosis, infection by a pathogen such as a virus including HIV-1, Hepatitis virus, a microorganism or a malarial parasite, congenital immunodeficiency, adverse reaction following bone marrow or tissue transplantation or chronic fatigue syndrome.

12. A diagnostic assay according to any one of aspects 1 to 11 wherein the molecules immobilized on the solid support are in an arrangement in the array such that upon interaction between the molecules and the binding partners, a differential pattern of density provides an identifiable signal.

13. A method for determining the presence of a disease condition or disorder or a propensity to develop a disease condition or disorder such as but not limited to cancer or a non-neoplastic disorder in an animal, avian species or plant, said method comprising obtaining a biological sample from said animal, avian species or plant comprising free binding partners or binding partners bound to a cell surface associated directly or indirectly with said disease condition or disorder and contacting said biological sample with a solid support comprising an array of molecules capable of binding to said binding partners wherein the pattern of interaction with the binding partners is indicative of the disease condition or disorder or a propensity to develop said disease condition or disorder.

erthematosis infection by a pathogen such as a virus including HIV-1, Hepatitis virus, a microorganism or a malarial parasitem congenital immunodeficiency, adverse reaction following bone marrow or tissue transplantation or chronic fatigue syndrome.

20. A method according to aspect 13 wherein the array comprises immunoglobulins in discrete regions of the solid support and the binding partners are antigens expressed on the surface of a normal or cancerous cell or are released by a normal or cancerous cell.

21. A method according to aspect 20 wherein the array comprises the formula:

$$\left[\left[q_{o_1} \right]_{e}^{m_1} \left[q_{o_2} \right]_{f}^{m_2} \cdots\cdots \left[q_{o_k} \right]_{g}^{m_i} \right]_{y}$$

wherein

q is an immunoglobulin specific for an antigen expressed on a normal cell or cancerous cell or antigen released by a normal cell or cancerous cell;

m_1 m_2 ..... m_i represent members of the same immunoglobulin group which bind to different parts of the same antigen;

o_1 o_2 ..... o_k represent different groups of immunoglobulins defined by specificity to different antigens.

e, f and g represent the number of different immunoglobulins within each of groups o_1 o_2 ..... o_k, respectively and wherein e, f and g may be the same or different and each is from 0 to 100 provided at least one of e, f or g is not 0;

y is the total number of groups of immunoglobulins on the array and is from about 2 to about 2000.

22. A method according to aspect 20 wherein the immunoglobulins are monoclonal antibodies.

23. A method according to aspects 21 or 22 wherein the immunoglobulins are specific for

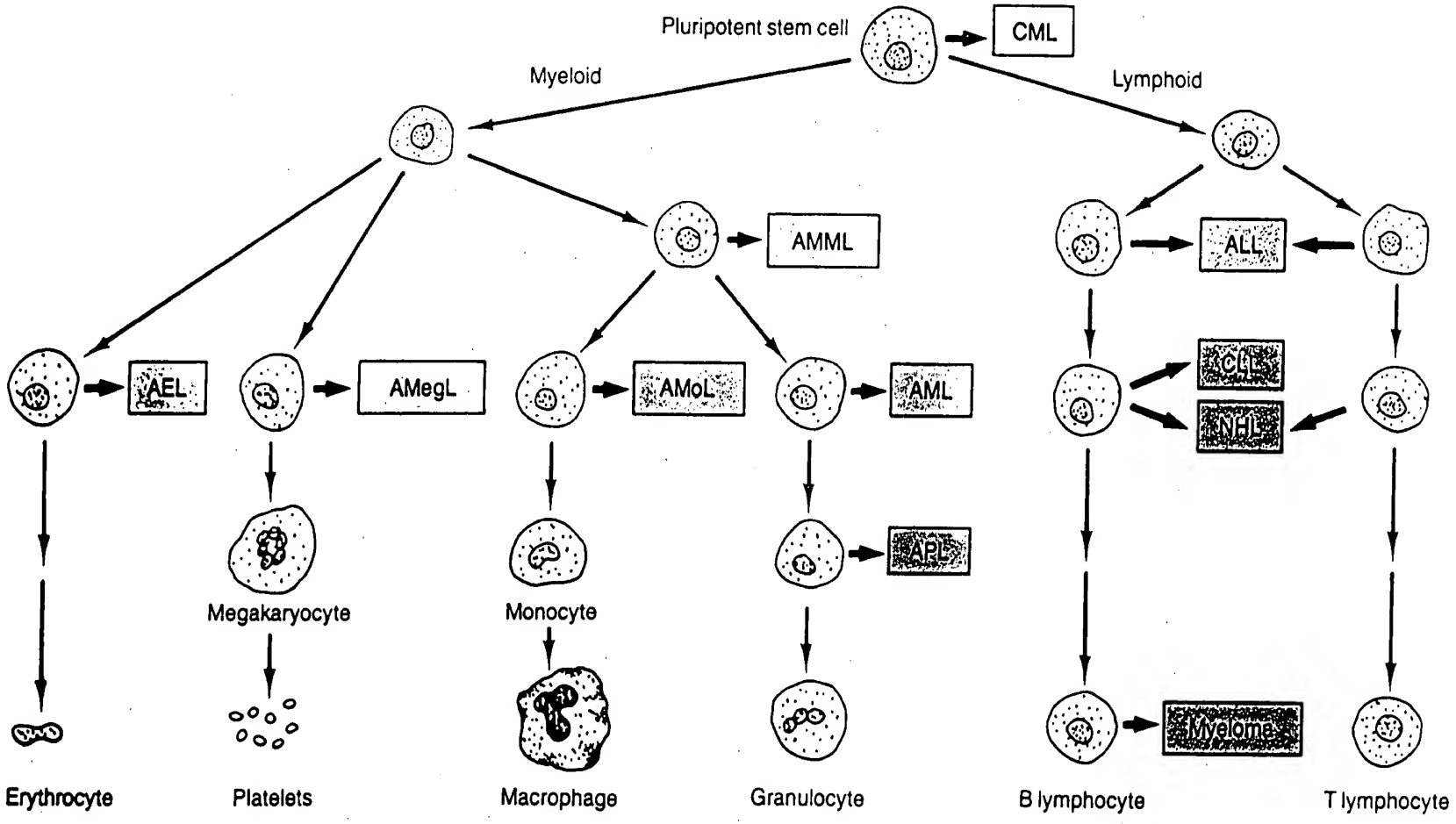

Pluripotent stem cell
CML
Myeloid
Lymphoid
AMML
ALL
AEL
AMegL
AMoL
AML
CLL
NHL
APL
Megakaryocyte
Monocyte
Myeloma
Erythrocyte
Platelets
Macrophage
Granulocyte
B lymphocyte
T lymphocyte

FIGURE 1

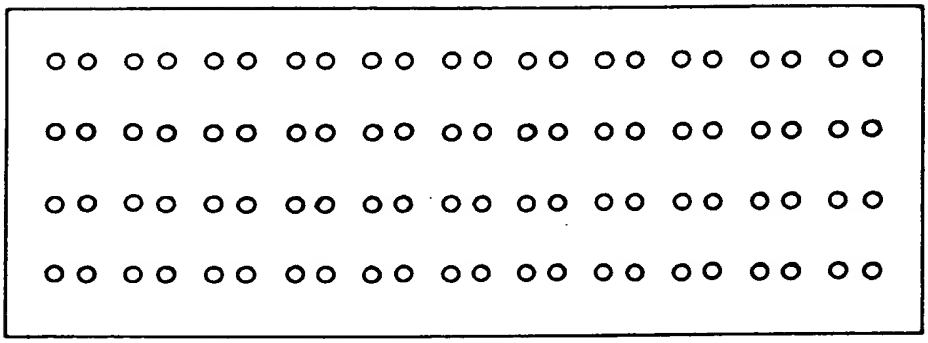


FIG 3a

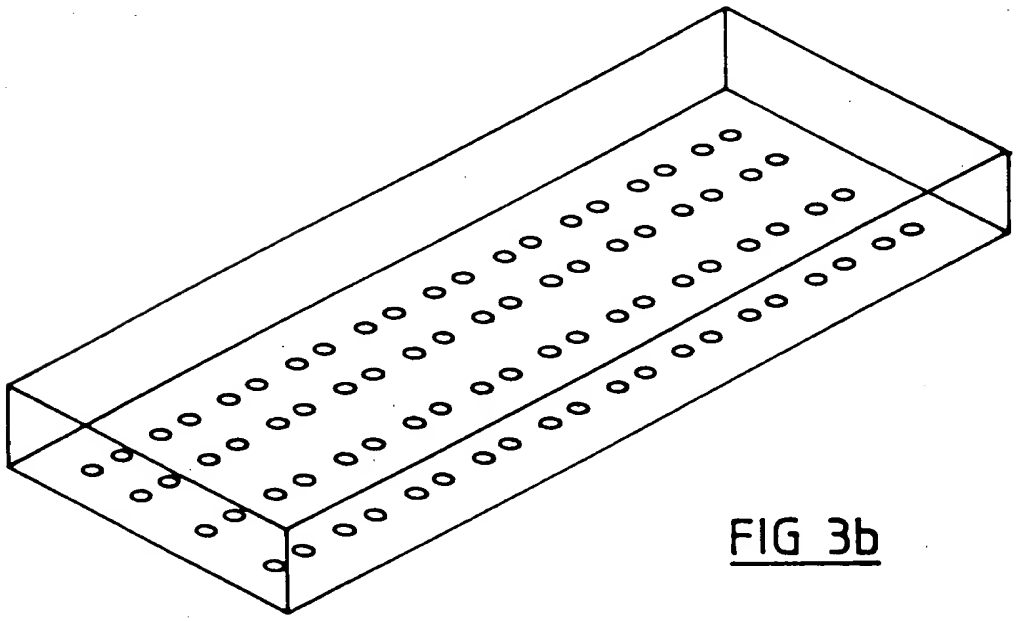
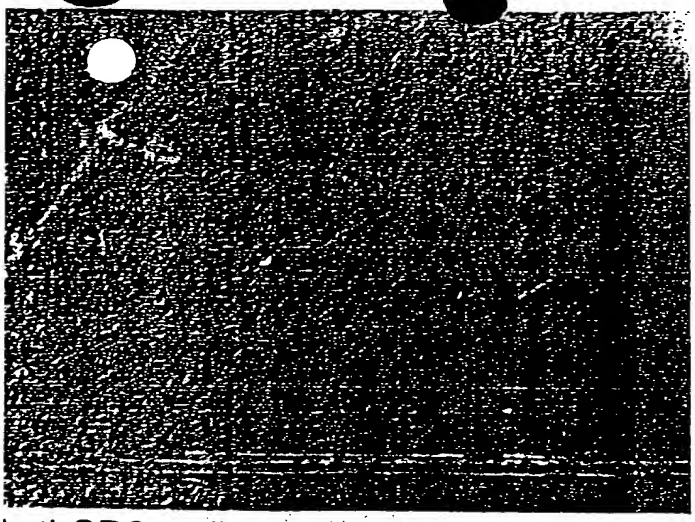


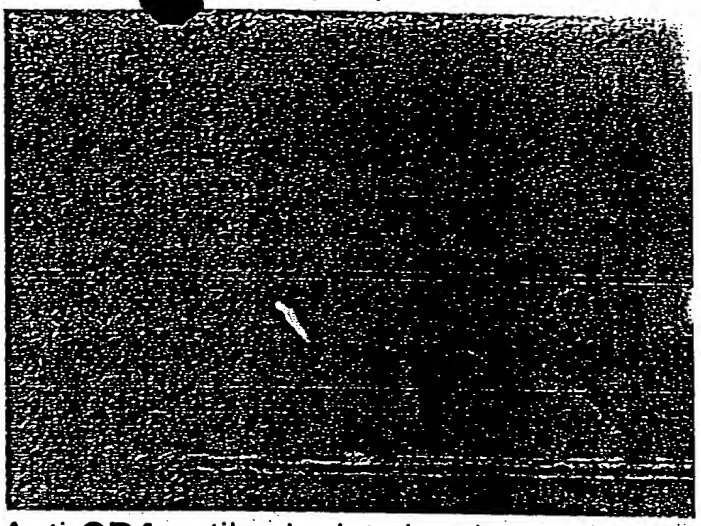
FIG 3b

N[redacted]ki Images
m[redacted]20

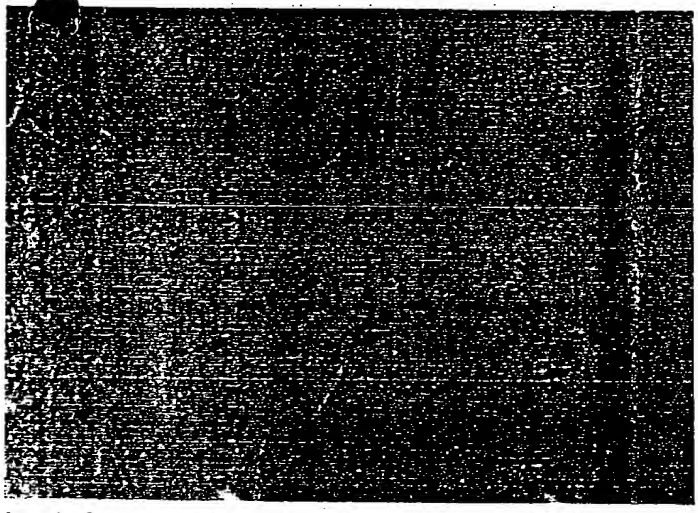
RAJI CELL ARRAY: B-cell Burkitt's Lymphoma



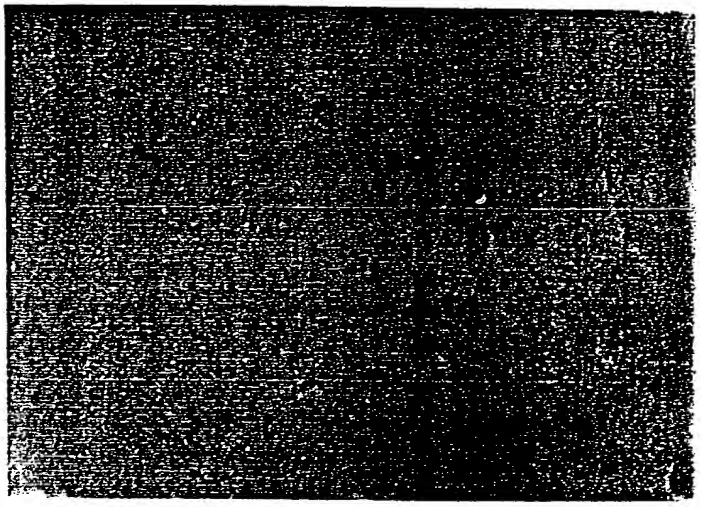
Anti-**CD3** antibody dot showing negative selection for Raji cells.



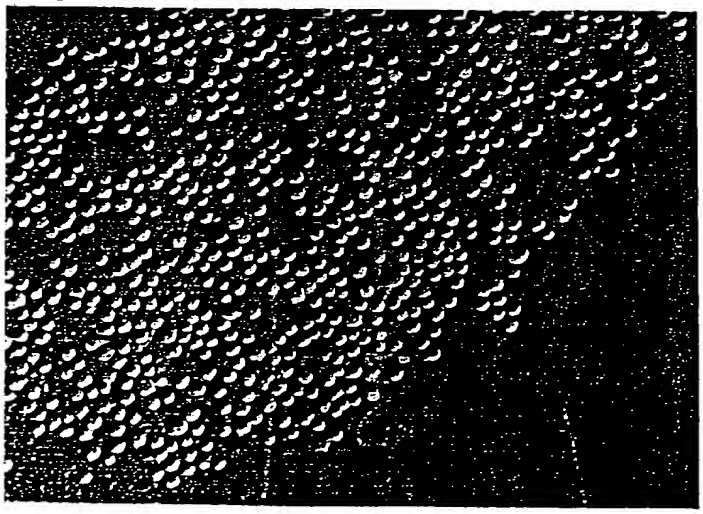
Anti-**CD4** antibody dot showing no binding of cells.



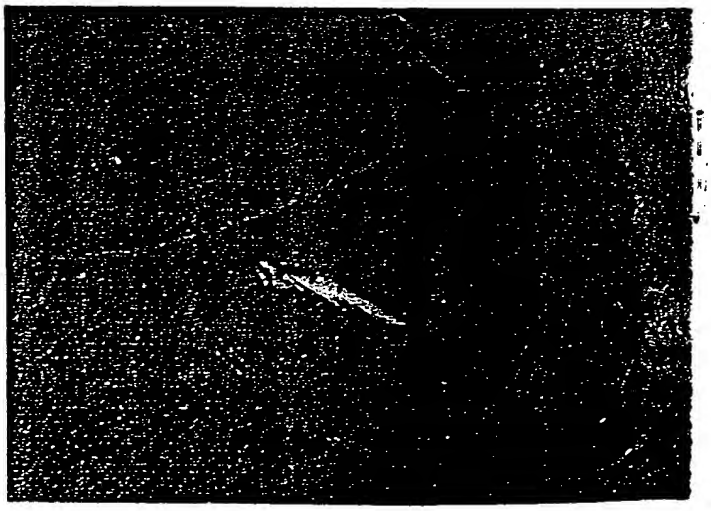
Anti-**CD8** antibody dot with no retention of cells.



Anti-**CD14** antibody dot showing non-selective binding of cells.



Anti-**CD19** antibody dot showing selectivity for Raji cells due to a high expression of surface CD19 antigen.



Negative expression of **CD56** surface antigen by Raji cells.

FIGURE 4B